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PHOTOADAPTATIONS OF THE CARIBBEAN COLONIAL ASCIDIAN-CYANOPHYTE SYMBIOSIS *TRIDIDEMNUM SOLIDUM*

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Abstract

Photosynthetic organisms show a number of photoadaptations which enable them to acclimate to the light regime in which they grow. In the case of invertebrate-algal symbioses, the responses can occur in both the algae and the invertebrate. Colonies of the colonial ascidian-cyanophyte symbiosis *Trididemnum solidum* at Galeta, Panama, show morphological variation relative to the light regime in which they live. Colonies growing in full sunlight are white, thicker, more heavily calcified, and distribute their cyanophytes more uniformly throughout the depth of the colony than do colonies in lower light regimes. Shaded colonies are purple, thinner, have fewer spicules at the colony surface, concentrate their algae near the surface, and have a greater amount of phycoerythrin relative to phycocyanin in their symbiotic algae. The purple coloration of low light colonies appears to be due primarily to the phycobilin pigment of the algae. Experimental shading of colonies in the lowest light areas in which they are found at Galeta, resulted in significantly higher mortality than colonies beneath clear control roofs. The ascidian host appears to be physiologically dependent upon its symbionts and capable of changing its morphology in response to ambient light levels.

Introduction

Symbioses with unicellular algae occur in a wide range of marine invertebrates from single cell protozoa (Karakashian, 1963) to the giant clams, *Tridacna* (Taylor, 1973). Most research on such symbioses has examined eucaryotic symbionts (zooxanthellae, zoochlorellae) in the tisues of anthozoan coelenterates and other invertebrates (reviewed by Trench, 1980). However, the occurrence of procaryotic symbionts has been noted in sponges (Sara, 1971; Vacelet, 1971; Wilkinson, 1982) and several species of ascidians (Lewin, 1975).

Although the existence of colonial ascidians with symbiotic algae has been known for over seventy years (Herdman, 1907), the fact that the symbionts are procaryotes was not realized until 1975 (Newcomb and Pugh, 1975). The algae had previously been mistakenly identified as zooxanthellae (Hastings, 1931; Smith, 1935) or zoochlorellae (Smith, 1935; Tokioka, 1967). After an extensive analysis of the algae associated with didemnid ascidians of the Pacific, Newcomb and Pugh (1975) concluded that "some or all of the zoochlorellae reported in ascidians by past workers may have been blue-green algae." This belief is corroborated by Lafargue and Duclaux (1979).

Lewin (1975) described a unique group of symbiotic algae found only in association with didemnid ascidians. These algae possess traits which do not conform to the current taxonomy of unicellular algae. They contain chlorophyll b, lack phycobilin pigments,

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and occasionally have stacked thylakoids (Thorne *et al.*, 1977; Giddings *et al.*, 1980). This suggests that they are eucaryotes, perhaps chlorophytes. However, they have a typical cyanophyte structure without a nuclear membrane or membranous organelles, and with a cell wall that has four layers (Whatley, 1977) and the peptidoglycan complex that characterizes procaryotes (Moriarty, 1979). Lewin (1977) has established the Prochlorophyta as a new division, with *Prochloron* as the type genus.

Of the 20 species of ascidians obligately associated with symbiotic algae (Kott, 1982), 17 possess algae of the newly described genus *Prochloron* (Lewin, 1975). The other three species (*Tridemnum solidum, T. Cyanophorum,* and *Didemnum viride*) contain symbionts of the genus *Synechocystis,* a true cyanophyte with phycobilin photosynthetic pigments (Lafargue and Duclaux, 1979; Kott, 1980). These three species range in color from grey to purple depending upon the light regime in which they grow (Olson, 1980).

The ecology and physiology of *Tridemnum solidum,* a species found only in the Caribbean, was investigated in Curacao and reported by Bak *et al.* (1981), van Duyl *et al.* (1981), and Sybesma *et al.* (1981). In this paper I present data on *T. solidum,* from Galeta, Panama, documenting the changes in photosynthetic pigments and colony morphology between high and low light environments, and the results of an experiment which demonstrates the obligate nature of this symbiosis. For simplicity, I have chosen to use the term "algae" to refer to the *Synechocystis* cells, even though they are actually cyanophytes.

Materials and Methods

Field research was conducted on the fore reef at Galeta, Panama, in front of the Galeta Marine Laboratory. The Galeta reef is adjacent to mangroves and has a heavy sediment load (Cubit and Williams, 1983). *T. solidum* occurs from the low intertidal zone to approximately 8 m depth around Galeta.

Colony morphology

In this paper the term colony is used to refer to a single specimen of *T. solidum* composed of physiologically attached zooids. Colonies were collected from a full sunlight habitat (less than 1 m depth, unshaded; these will be referred to as "light colonies") and a shade habitat (2 m depth, shaded by overhanging ledge; referred to as "shade colonies"). A 0.1 m^2 quadrat was placed over a large aggregation of colonies, then starting at one side, all colonies were collected until 50 colonies were obtained. Colonies were gently peeled off the coral rubble on which they grew. A few colonies had fragments of rubble or algae attached to their tunic, but these were easily cleaned off. To measure upper surface area, colonies were laid flat on paper and outlined, then the outline was measured with a planimeter. Colony volume was measured by displacement of seawater in a graduated cylinder. Colonies were dried at 60°C for 24 hours and weighed. To obtain ash-free dry weights, colonies were combusted in a muffle furnace at 500°C for 12 hours and weighed.

Histology

Colonies were fixed in 5 percent Bouins solution and kept for 3 months, during which time they decalcified. Specimens were embedded in paraffin and 5 μm sections were cut. Sections were stained with hematoxylin and fast green. Algal distribution "transects" were performed by examining slides under a compound microscope with

an ocular grid. Three randomly selected transects were performed on one section each from four light colonies and four shade colonies.

No attempt was made to quantify algal cell densities in terms of surface area of colony or biomass. This is because the algae are firmly embedded in the tunicin-mucopolysaccharide matrix of the tunic. Maceration and homogenization failed to produce any reliable separation of algal and animal tissue.

Photosynthetic pigments

Specimens for pigment extraction were frozen at −20°C and transported to Cambridge, Massachusetts, in dry ice kept in total darkness. Colonies were sampled by cutting a square core of 1 cm × 1 cm from the top of the colony to the bottom using a razor blade. Extractions were performed for chlorophylls and phycobilins using separate samples for each. The squares were ground to a powder in a mortar and pestle with dry ice. Phycobilin pigments were extracted in 10 ml of 0.01 *M* $NaHPO_4$, 0.15 *M* NaCl, pH = 7.0. Chlorophyll *a* and carotenoids were extracted in 25 ml of 90% acetone. The samples were centrifuged at 14,000 rpm for 20 minutes, then analyzed on a Cary 219 scanning spectrophotometer. Absorbance maxima for phycoerythrin and phycocyanin were read at 545 and 615 nm, respectively. Phycobilin pigment concentrations were calculated with the equations of Bennett and Bogorad (1973). Chlorophyll *a* concentration and total carotenoids were calculated using the equations of Jeffrey and Humphrey (1975), and Strickland and Parsons (1965), respectively.

Shading experiment

To determine whether *T. solidum* is physiologically dependent upon its symbiotic algae, the supply of sunlight to colonies was altered *in situ* using ¼" thick plexiglas roofs. Six pairs of 20 cm by 20 cm roofs, consisting of one clear roof (control) and one black roof (shade) were mounted approximately 2 cm above the substratum. The locations chosen were areas of near 100 percent space cover by *T. solidum* and were at the very lowest light intensities that *T. solidum* is found at Galeta (approx. 5 m depth).

The roofs were fastened to the substratum with one nail at each corner attached with plastic cable ties. The area beneath each roof was monitored at the beginning, then after four and six days by removing the roof, and overlaying a piece of clear plexiglas with 100 random dots. Each dot was scored for the presence or absence of *T. solidum* beneath it. The experiment was frequently checked for the presence of organisms seeking refuge beneath the dark roofs, but none was observed.

Results

Colony morphology

Colonies of *T. solidum* on the fore reef at Galeta exhibit a continuum of colors from white to gray to purple (Figs. 1, 2). The coloration of colonies closely corresponds to the habitat in which the colonies live. Colonies in full sunlight, shallow sites were bright white and bulbous, packed with calcareous spicules (Fig. 3), to the top of the colony and slightly larger (Fig. 4) than shaded colonies. Colonies in shaded habitats were dark purple, with few spicules in the top 1 mm of the tunic. The thickness of light colonies ($\bar{x}$ = 3.28 mm, S.E. = 0.088, n = 50) was significantly greater than shade colonies ($\bar{x}$ = 2.91 mm, S.E. = 0.083, n = 50) (*t*-test, $P < 0.005$).

The difference in calcification between shade and light colonies is shown by the difference in ash-free dry weights. Light and shade colonies had mean percent ash-

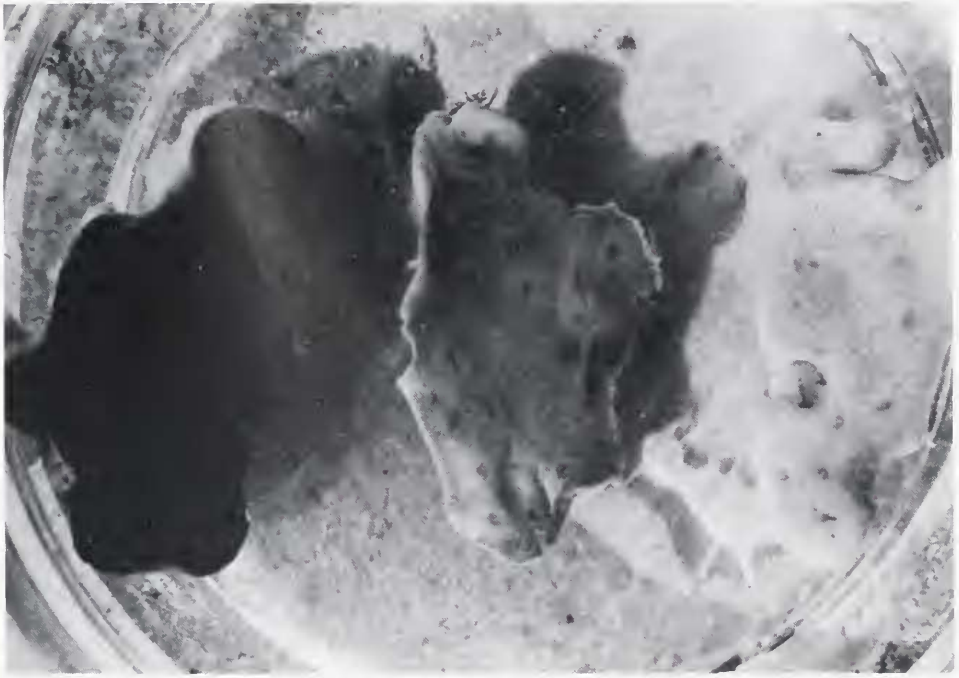

FIGURE 1. Top view of *Tridemnum solidum* colonies collected from (left to right) low, medium, and high light intensity habitats. Colonies are approximately 10 cm in length.

FIGURE 2. Colonies in a full sunlight habitat (<1 m depth) at midday. Note oxygen bubbles trapped within tunic. Small white specs on bubbles are calcareous spicules.

FIGURE 3. Scanning electron micrograph of calcareous, stellate spicules and *Synechocystis* algae (small spheres) in the tunic of *T. solidum*. Top portion of photo is top of colony. (Photo by E. Seling.)

free dry weights (ash-free dry weight/total dry weight) of 13.28 (S.D. = 1.42; n = 9) and 15.46 (S.D. = 1.52; n = 9), respectively. Thus light colonies have a significantly greater inorganic component (t-test, $P < 0.01$). This difference is also reflected in the ratio of volume to dry weight (Table I) which shows that light colonies have a greater density than shade colonies. It appears that much of the difference could be accounted for by the top layer of the colonies. Very few, if any, spicules can be seen in the top 2–3 mm of shade colonies, whereas light colonies are generally packed to the top with spicules. Cross sections revealed a zone at the top of light colonies with very little algae (Fig. 5).

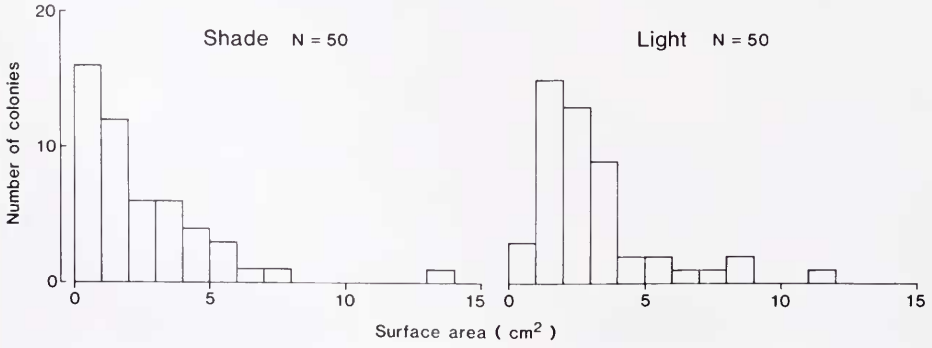

FIGURE 4. Size distribution, by surface area, of colonies from shade and full sunlight locations.

TABLE I

Regression analysis of colony morphology parameters. Comparison of slopes and elevations performed with t-*test (Zar, 1974)*

Regression (y *vs.* x)	Light				Shade				Level of significance	
	n	Slope	Y-intercept	r^2	n	Slope	Y-intercept	r^2	Slopes	Y-int.
y x										
Volume *vs.* dry weight	50	1.80	0.014	0.91	50	1.82	0.132	0.87	N.S.	$P < 0.005$
Volume *vs.* upper surface area	50	0.187	0.029	0.80	50	0.134	0.110	0.86	$P < 0.0005$	N.S.
Upper surface *vs.* dry weight	50	8.50	0.314	0.89	50	13.15	0.233	0.96	$P < 0.01$	$P < 0.01$

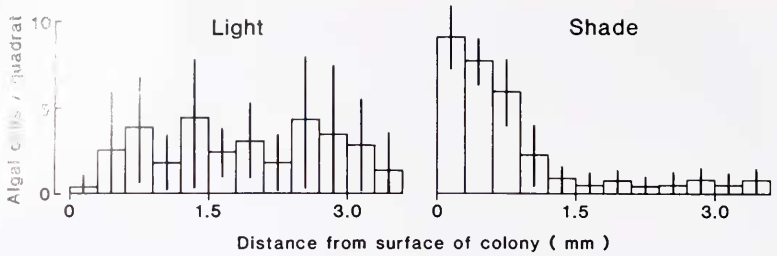

FIGURE 5. Distribution of algae through depth of colonies from sections of shade versus full sunlight colonies. n = 3 transects from 4 colonies each. Error bars are standard errors. Algal abundance is expressed as a relative measure (# cells/quadrat).

Photoadaptation

The difference in coloration of colonies (Fig. 1) is primarily due to their levels of phycobilin pigments. The algae of dissected light colonies are light brown in color, those of shade colonies are dark purple, almost black. There was twice as much phycoerythrin per unit of surface area in shade colonies than in sun colonies (Table II). Aqueous extracts of shade colonies were red in color *versus* light pink for sunlight colonies. Shade colonies also had a greater mean concentration of phycocyanin than light colonies.

In addition to the differences in the amount of phycobilin pigment, extracts from the sun colonies had much greater absorbance in the ultraviolet-B region, with a peak absorbance around 330 nm. The difference in absorbance down to 400 nm can be seen in Figure 6.

Shading experiment

During the six days of the shading experiment, colonies beneath the dark roofs contracted and most began to deteriorate. Of the approximately 100 colonies beneath

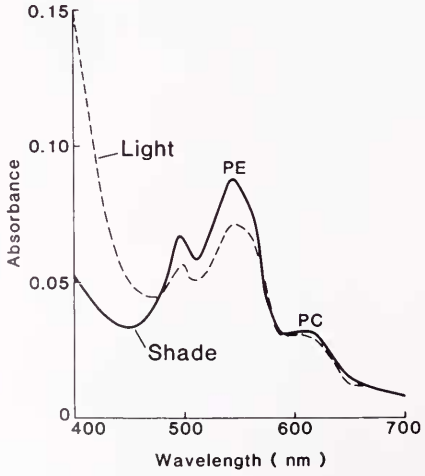

FIGURE 6. Absorbance spectra of water soluble pigments from representative light and shade colonies. PC—phycocyanin, PE—phycoerythrin.

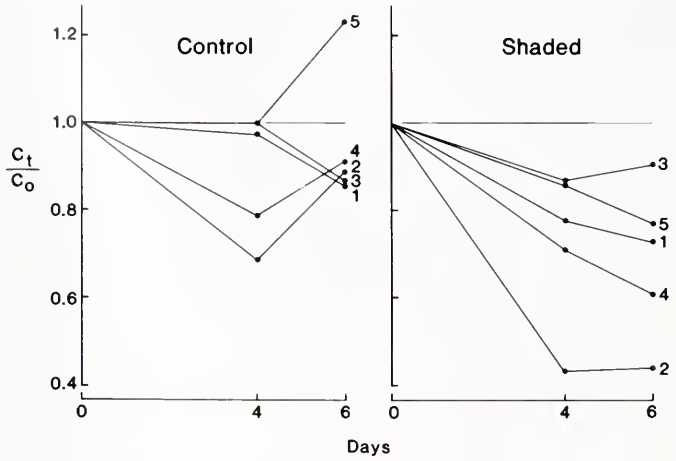

FIGURE 7. Results of shading experiment. Data are % cover of *Tridemnum solidum* at time t (C_t) divided by initial % cover (C_0). Treatments were paired according to numbers.

the dark roofs, twelve entire colonies died. No sign of tissue death was observed in the colonies beneath the clear roofs. The mean relative change in percent cover of colonies beneath the shade treatments (−31.1%, S.D. = 0.17) was significantly greater than the relative change in percent cover of the colonies beneath the controls (−4.9%, S.D. = 0.16) ($P < 0.05$, Wilcoxon paired sampled test) (Fig. 7).

As colonies began to die beneath the shades, they turned green for a day or two, then white. The change to green is most likely due to the breakdown of the phycobilin pigments, removing the purple coloration. The sky was very cloudy during the experiment, which might account for the decline in percent cover of the control colonies. The breakdown of phycobilin pigments in stressed algae is documented for laboratory cultures of blue-green algae such as *Anabaena* (Ownby *et al.*, 1979) and *Synechococcus* (Yamanaka and Glazer, 1980). The white material remaining after about 3 days was primarily spicules.

DISCUSSION

Photoadaptation

Photosynthetic organisms acclimate to the light regime in which they grow primarily through changes in: (1) the production of photosynthetic pigments, (2) the structure of chloroplasts, and (3) the photosynthetic response (Boardman, 1977). Studies of photoadaptation in invertebrate-algal symbioses have focused on eucaryotic cells, particularly the dinoflagellates found in symbiosis with corals (Titlyanov *et al.*, 1980; Zvalinskii *et al.*, 1980; Falkowski and Dubinsky, 1981; Dustan, 1982; Chalker *et al.*, 1984; Kinzie *et al.*, 1984; Porter *et al.*, 1984).

Tridemnum solidum colonies from full sunlight *versus* shaded habitats at Galeta showed the following five differences:

(1) *Distribution of algae within colony*

Algae in the tunic of shade colonies occurred primarily in the top 1 mm of the colony (Fig. 5). This packing of algae near the surface appears to be analogous to the arrangements of chloroplasts in cells of shaded-adapted terrestrial plants (Boardman,

1977). Algae located at the top of the colony would be exposed to the maximum level of light available. The mechanism through which the algae arrive at this location is unknown.

(2) *Ratio of organic to inorganic matter*

Shade colonies have a greater proportion of organic matter, as shown by the ratio of ash-free dry weight to dry weight. Light colonies have more spicules and are more dense (Table I). This same response has been reported for other colonial ascidian-algal associations. Kott (1980) reported that colonies of *Lissoclinum voeltzkowi* on the Great Barrier Reef produce a more dense layer of spicules at the surface of the colony when growing in full sunlight than when growing in the shade. *Didemnum viride* (another ascidian-*Synechocystis* association) also shows less dense spicules in shaded habitats (pers. obs.). Newly settled larvae of *Didemnum molle* produce few spicules if shaded, but produce a dense layer of spicules if grown in full sunlight (Olson, 1984).

There are at least three reasonable hypotheses as to why spicule production is directly related to sunlight intensity. Spicule production might be (1) an ecological response to predators because high light intensity habitats are usually exposed to a wider variety of predators, or (2) it might be used to regulate the amount of sunlight entering the colony, or (3) it could be a physiological consequence of photosynthesis by symbiotic algae. These hypotheses are not mutually exclusive.

Field observations suggest that predation might be an important factor. *T. solidum* colonies grow in fully exposed habitats at Galeta, yet no fish were ever observed to prey on them. The only predators ever observed were a flatworm and occasionally, the sea urchin *Diadema antillarum.*

Spicules in the surface layer must function to some extent to reduce the amount of sunlight entering the colony. The distribution of algae through the depth of the colony (Fig. 5) suggests that light levels in full sunlight habitats might be super-saturating. The presence of spicules and U.V. absorbing compounds might protect the algae. In shaded habitats, having spicules in the top of the colony would reduce light penetration, shading algae below.

The third hypothesis was postulated for corals by Goreau (1959). Light enhanced calcification has been documented extensively for corals (see Chalker, 1983, for review). Increased spicule production in *T. solidum* growing in full sunlight might simply be a physiological consequence of the ambient light regime. However, among the ascidian-algal symbioses there are three species, all in the genus *Diplosoma,* which lack spicules despite the presence of symbiotic algae (Kott, 1980). There are also a wide range of didemnid ascidians which lack symbiotic algae, yet possess calcareous spicules (Eldredge, 1967). This casts doubt on the physiological consequence hypothesis.

(3) *Photosynthetic pigments*

The most significant, and obvious, difference in photosynthetic pigments between light and shade colonies was the phycoerythrin content (Table II). Phycoerythrin is an accessory photosynthetic pigment which has an absorbance maximum in the green light region around 560 nm (Bennett and Bogorad, 1973). Increased production of this pigment in the symbiotic algae of shade colonies follows the classic chromatic adaptation of blue-green algae described by Fujito and Hattori (1960, 1962). Cyanophytes grown in green light produce greater amounts of phycoerythrin. Those grown in red light produce more of the red light absorbing pigment phycocyanin. This results in different coloration of the algae growing in different light spectra, as appears to be the case with the *Synechocystis* of *T. solidum.*

TABLE II

Mean concentrations of photosynthetic pigments per unit area of T. solidum *colonies from light and shade habitats*

	Light		Shade		Level of significance*
Chlorophyll a ($\mu g/cm^2$)	98.73	(20.89)	110.65	(12.85)	N.S.
Total Carotenoid ($\mu g/cm^2$)	26.08	(4.71)	23.13	(2.97)	N.S.
Carotenoid/Chlorophyll *a*	0.27	(0.03)	0.21	(0.006)	$P < 0.001$
Phycocyanin ($\mu g/cm^2$)	69.0	(14.48)	105.0	(34.25)	$P < 0.05$
Phycoerythrin ($\mu g/cm^2$)	68.3	(16.56)	155.0	(39.9)	$P < 0.001$
Phycocyanin/Phycoerythrin	1.02	(0.12)	0.67	(0.14)	$P < 0.005$

Values in parentheses are standard deviations. n = 6 colonies for all values. *t-test.

Kinzie *et al.* (1984) have emphasized an important distinction in photoadaptation; whether the response is to light quality (spectral composition) or light intensity. Since dark purple *T. solidum* colonies can be found in shaded sites at shallow depths (1–2 m) and colonies in unshaded habitats at deeper depth (4–5 m) have the light appearance, it would appear that the responses are largely to decreased light intensity. This aspect awaits further investigation.

The amount of chlorophyll *a* per cm^2 was slightly greater in shade colonies (Table II) although the difference was not significant due to the large variation. Shade-adapted algae generally have a greater amount of chlorophyll *a* per cell (Porter *et al.*, 1984). However, Sybesma *et al.* (1981) also found no significant difference in chlorophyll *a* content of colonies growing at different light levels. The values I obtained for chlorophyll *a* were approximately four times greater than those found by Sybesma *et al.* (1981) for *T. solidum* in Curacao, and are approximately ten times greater than values reported by Porter *et al.* (1984) for corals.

Although the total amount of carotenoids did not differ between light and shade colonies (Table II), there was a significantly higher ratio of total carotenoids/chlorophyll *a* for light colonies. This is consistent with what is known for terrestrial plants, in which it is suggested that carotenoids might serve to protect chlorophyll *a* from photodegradation (Robertson *et al.*, 1966).

(4) *Colony size and thickness*

Colonies in full sunlight were slightly larger (Fig. 2) and thicker. This change in colony morphology resembles the variation with depth that is seen with reef-building corals (Dustan, 1975; Redalje, 1976; Foster, 1979) in which colonies from greater depth, growing in lower light, assume a thinner, more plating morphology.

(5) *U.V. absorbing compounds*

Though ultraviolet radiation penetrates little more than the first 10 m of the ocean (Jerlov, 1968), it is known to inhibit photosynthesis (Smith *et al.*, 1980) and can be lethal to invertebrates in shallow water (Jokiel, 1980). *T. solidum* at Galeta is abundant in exposed areas all the way up to the low tide level. Light colonies experience almost the full level of surface U.V. irradiation and thus must somehow be protected from this. The aqueous extracts of colonies (Fig. 6) showed the presence of U.V. absorbing compounds in much greater quantity in light colonies than shade. This peak had a maximum at approximately 330 nm, the upper end of the U.V.-B region (Smith and Baker, 1979). The compound or compounds responsible for the absorption probably

function in a manner similar to the "S-320" substances isolated from corals (Shibata, 1969).

The importance of sunlight

The shading experiment (Fig. 7) indicates that the ascidian host is dependent upon the well-being of its symbiotic algae. Reduction of light, beyond a certain level, is lethal to the colony. Whether the death of the colony is caused by starvation from insufficient production by the algae, or is due to toxicity of the dying algae, is unclear. Regardless, both symbionts are unable to survive without sunlight. Thus the distribution of *T. solidum* should be limited to habitats in which sunlight is in excess of the minimum level needed for the symbiotic algae. On the other hand, there does not appear to be any maximum light level at which colonies can survive since they occur in the low intertidal, unshaded, on the Galeta reef flat.

Considerable data already exist on the ecology and physiology of *Tridemnum solidum* in Curacao (Bak *et al.*, 1981; Duyl *et al.*, 1981; Sybesma *et al.*, 1981). It is important to note the substantial differences (particularly colony size) between that population and the population of Galeta, Panama. Colonies from Curacao are as large as 50 cm in diameter. Almost all of the colonies reported by Bak *et al.* (1981) were at least 15 cm diameter, or about 175 cm^2 surface area (assuming a circular shape). In contrast, Panama colonies were rarely more than 10 cm in length, and the surface area of the largest colony collected for the colony morphology analysis was 14 cm^2 (Fig. 2).

Colonies of *T. solidum* at Galeta divide very rapidly (taking only one day at times) producing a "jigsaw puzzle" of thousands of colonies abutting each other, and covering areas of several square m. Whereas a single Curacao colony might cover 0.25 m^2, at Galeta I was able to collect all 50 colonies for the colony morphology measurements in less than 0.1 m^2 area.

Although both populations have been identified by R. H. Millar as *T. solidum* (Cubit and Williams, 1983; Duyl *et al.*, 1981), there appear to be differences in their reproductive patterns. In Curacao, colony fusion occurs almost as frequently as colony division, so there is little asexual propagation (Bak *et al.*, 1981). Although I watched closely for it, I never observed colony fusion in *T. solidum* at Galeta. More than 50% of all colonies produced larvae throughout the year in Curacao (Duyl *et al.*, 1981). At Galeta, Millar (1974) found that for half of the year (January–June) no colonies produced larvae.

The difference in colony size between the Galeta population and the populations in Curacao (Bak *et al.*, 1981), Puerto Rico, and St. Croix (pers. obs.) poses an interesting question. Is this difference due to a divergence in life history patterns, with colonies in the Galeta population maximizing the number of distinct colonies within a genetic individual? Or is there a physiological reason for colonies maintaining a small size, such as hydrodynamics (Vogel, 1981) or food availability (Sebens, 1979)? The first response of the shaded colonies, in the shading experiment, was to shrink and divide. Stoner (1985) found that colonies of the ascidian-*Prochloron* symbiosis *Diplosoma similis* divided at smaller sizes when grown at low light levels. Water clarity at Galeta for much of the year is considerably lower than for the islands of Curacao, Puerto Rico, and St. Croix. The Galeta reef, being adjacent to mangroves, has a heavy sediment load and water clarity is extremely low for most of the dry season [December to April (Millar, 1974)]. Reduced light penetration would reduce the productivity of the symbiotic algae, possibly reducing the supply of energy available to the ascidian host. Perhaps the more stressful physical conditions at Galeta have selected for a morph of *T. solidum* with colony division at a smaller size.

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LITERATURE CITED

BAK, R. P. M., J. SYBESMA, AND F. C. VAN DUYL. 1981. The ecology of the tropical compound ascidian *Tridemnum solidum* (Van Name). II. Abundance, growth, and survival. *Mar. Ecol. Prog. Ser.* **6**: 43–52.

BENNETT, A., AND L. BOGORAD. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell. Biol.* **58**: 419–435.

BOARDMAN, R. K. 1977. Comparative photosynthesis of sun and shade plants. *Ann. Rev. Plant Physiol.* **28**: 355.

CHALKER, B. E. 1983. Calcification by corals and other animals on the reef. Pp. 29–45 in *Perspectives on Coral Reefs*, D. J. Barnes, ed. Aust. Inst. Marine Science.

CHALKER, B. E., T. COX, AND W. C. DUNLAP. 1984. Seasonal changes in primary production and photoadaptation by the reef-building coral *Acropora granulosa* on the Great Barrier Reef. Pp. 73–84 in *Marine Phytoplankton and Productivity*, O. Holm-Hansen, L. Bolis, and R. Gilles, eds. Springer-Verlag, Berlin.

CUBIT, J., AND S. WILLIAMS. 1983. The invertebrates of Galeta Reef (Caribbean Panama): species list and bibliography. *Atoll Res. Bull.* **269**: 1–45.

DUSTAN, P. 1975. Growth and form in the reef-building coral *Montastrea annularis*. *Mar. Biol.* **33**: 101–107.

DUSTAN, P. 1982. Depth-dependent photoadaptation of the reef coral *Montastrea annularis*. *Mar. Biol.* **68**: 253–264.

VAN DUYL, F. C., R. P. M. BAK, AND J. SYBESMA. 1981. The ecology of the tropical compound ascidian *Tridemnum solidum*. I. Reproductive strategy and larval behavior. *Mar. Ecol. Prog. Ser.* **6**: 35–42.

ELDREDGE, L. G. 1967. A taxonomic review of Indo-Pacific didemnid ascidians and descriptions of twenty-three central Pacific species. *Micronesica* **2**: 161–261.

FALKOWSKI, P. G., AND Z. DUBINSKY. 1981. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. *Nature* **289**: 172–174.

FOSTER, A. B. 1979. Phenotypic plasticity in the reef corals *Montastrea annularis* (Ellis and Solander) and *Siderastrea siderea*. *J. Exp. Mar. Biol. Ecol.* **39**: 25–54.

FUJITO, Y., AND A. HATTORI. 1960. Effect of chromatic lights on phycobilin formation in a blue-green alga, *Tolypothrix tenuis*. *Plant Cell Physiol.* **1**: 293–303.

FUJITO, Y., AND A. HATTORI. 1962. Photochemical interconversion between precursors of phycobilin chromoproteids in *Tolypothrix tenuis*. *Plant Cell Physiol.* **3**: 209–220.

GIDDINGS, T. H., N. W. WITHERS, AND L. A. STAEHELIN. 1980. Supramolecular structures of stacked and unstacked regions of the photosynthetic membranes of *Prochloron* sp., a prokaryote. *Proc. Natl. Acad. Sci. U.S.A.* **77**: 352–356.

GOREAU, T. F. 1959. The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. *Biol. Bull.* **116**: 59–75.

HASTINGS, A. B. 1931. Tunicata. *Great Barrier Reef Expedition 1928–29 Sci. Rep.* **4**: 69–109.

HERDMAN, W. A. 1907. On the tunicata. *Ceylon Pearl Oyster Fish.* **5**: 295–348.

JEFFREY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1, and c2 in higher plants, algae, and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**: 191–194.

JERLOV, N. G. 1968. *Optical Oceanography*. Elsevier, New York. 194 pp.

JOKIEL, P. J. 1980. Solar ultraviolet radiation and coral reef epifauna. *Science* **207**: 1069–1071.

KARAKASHIAN, S. J. 1963. Growth of *Paramecium bursaria* as influenced by the presence of algal symbionts. *Physiol. Zool.* **36**: 52–68.

KINZIE, R. A., P. L. JOKIEL, AND R. YORK. 1984. Effects of light of altered spectral composition on coral zooxanthellae associations and on zooxanthellae *in vitro*. *Mar. Biol.* **78**: 239–248.

KOTT, P. 1950. Algal-bearing didemnid ascidians in the Indo-West Pacific. *Mem. Queensl. Mus.* **20:** 1–47.
KOTT, P. 1982. Didemnid-algal symbioses: host species in the Western Pacific. *Micronesica* **18:** 95–127.
LAFARGUE, F., AND G. DUCLAUX. 1979. Premier example en Atlantique tropical d'une association symbiotique entre une ascidie didemnidae et une cyanophycee chroococale: *Trididemnum cyanophorum* nov. sp. et *Synechocystis trididemni* nov. sp. *Ann. Inst. Oceanogr. Paris* **55:** 163–184.
LEWIN, R. A. 1975. A marine *Synechocystis* (Cyanophyta, Chroococale) epizoic on ascidians. *Phycologia* **4:** 153–160.
LEWIN, R. A. 1977. *Prochloron,* type genus of the Prochlorophyta. *Phycologia* **16:** 217.
MILLAR, R. H. 1974. A note on the breeding season of three ascidians on coral reefs at Galeta in the Caribbean Sea. *Mar. Biol.* **28:** 127–129.
MORIARTY, D. J. W. 1979. The presence of muramic acid in the cell wall of *Prochloron. Arch. Microbiol.* **120:** 191–193.
NEWCOMB, E. H., AND T. D. PUGH. 1975. Blue-green algae associated with ascidians of the Great Barrier Reef. *Nature* **253:** 533–534.
OLSON, R. R. 1980. Sun-shade adaptation of a colonial ascidian with a procaryotic symbiont. *Am. Zool.* **20:** 258.
OLSON, R. R. 1984. The life history and larval ecology of the ascidian-algal symbiosis *Didemnum molle.* Ph.D. dissertation, Harvard University, Cambridge.
OWNBY, J. D., M. SHANNAHAN, AND E. HOOD. 1979. Protein synthesis degradation in *Anabaena* during nitrogen synthesis. *J. Gen. Microbiol.* **110:** 255–261.
PORTER, J. W., L. MUSCATINE, Z. DUBINSKY, AND P. G. FALKOWSKI. 1984. Primary production and photoadaptation in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata. Proc. R. Soc. Lond.* **222:** 161–180.
REDALJE, R. 1976. Light adaptation strategies of hermatypic corals. *Pac. Sci.* **30:** 212.
ROBERTSON, D. S., D. D. BACHMANN, AND I. C. ANDERSON. 1966. Role of carotenoids in protecting chlorophyll from photodestruction. II. Studies on the effect of four modifiers of the albina cl_1 mutant of maize. *Photochem. Photobiol.* **5:** 797.
SARA, M. 1971. Ultrastructural aspects of the symbiosis between two species of the genus *Aphanocapsa* (Cynaophyceae) and *Ircinia variabilis* (Demospongiae). *Mar. Biol.* **11:** 214–221.
SEBENS, K. P. 1979. The energetics of asexual reproduction and colony formation in benthic marine invertebrates. *Am. Zool.* **19:** 683–697.
SHIBATA, K. 1969. Pigments and a UV-absorbing substance in corals and a blue-green algae living in the Great Barrier Reef, Australia. *Plant Cell Physiol.* **10:** 325–335.
SMITH, H. G. 1935. On the presence of algae in certain Ascidiacea. *Ann. Mag. Nat. Hist.* **15:** 615–626.
SMITH, R. C., K. S. BAKER, O. HOLM-HANSEN, AND R. OLSON. 1980. Photoinhibition of photosynthesis in natural waters. *Photochem. Photobiol.* **31:** 585–592.
STONER, D. S. 1985. The effects of light intensity on the morphology and life-history of the symbiotic colonial ascidian *Diplosoma similis. Proc. 5th Intl. Coral Reef Congress* **1:** 363.
STRICKLAND, J. D. H., AND T. F. PARSONS. 1965. A manual of seawater analysis. Second edition. *Bull. Fish. Res. Bd. Canada.* 203 pp.
SYBESMA, J., F. C. VAN DUYL, AND R. P. M. BAK. 1981. The ecology of the tropical compound ascidian *Trididemnum solidum* (Van Name). III. Symbiosis with unicellular algae. *Mar. Ecol. Prog. Ser.* **6:** 53–59.
TAYLOR, D. L. 1973. The cellular interations of algal-invertebrate symbiosis. *Adv. Mar. Biol.* **11:** 1–56.
THORNE, S. W., E. H. NEWCOMB, AND C. B. OSMOND. 1977. Identification of chlorophyll b in extracts of procaryotic algae by fluorescence spectroscopy. *Proc. Natl. Acad. Sci.* **74:** 575–578.
TOKIAKA, T. 1967. Pacific tunicata of the United States National Museum. *Bull. U. S. Natl. Mus.* **251:** 1–247.
TITLYANOV, E. A., M. G. SHAPOSHNIKOVA, AND V. I. ZVALINSKII. 1980. Photosynthesis and adaptation of corals to irradiance 1. Contents and native state of photosynthetic pigments in symbiotic microalga. *Photosynthetica* **14:** 413–421.
TRENCH, R. K. 1980. The cell biology of plant-animal symbiosis. *Ann. Rev. Plant Physiol.* **30:** 485–531.
VACELET, J. 1971. Etude en microscopie electronique de l'association entre une cyanophycee chroococale et une sponge du genre *Verongia. J. Microscopie* **12:** 363–380.
VOGEL, S. 1981. *Life in Moving Fluids: the Physical Biology of Flow.* Willard Grant Press, Boston. 352 pp.
WHATLEY, J. 1977. The fine structure of *Prochloron. New Phytol.* **79:** 309–313.
WILKINSON, C. 1982. Net primary productivity in coral reef sponges. *Science* **219:** 410–412.
YAMANAKA, G., AND A. M. GLAZER. 1980. Dynamic aspects of phycobilisome structure. *Arch. Microbiol.* **124:** 29–47.
ZVALINSKII, V. I., V. A. LELETKIN, E. A. TITLYANOV, AND M. A. SHAPOSHNIKOVA. 1980. Photosynthesis and adaptation of corals to irradiance in oxygen exchange. *Photosynthetica* **14:** 422–430.